January 22, 1948.

Dear Ed-

I've just run through a prep. on W-94 with, I am sorry to say, no signs of successful transformation. You may be interested in the prep. W-94 was grown in NZCase 1%, Phosphate buffer .4%, glucose ½% in 12 l. aerated in a 5gal. carboy. 53 g. Sharples paste was recovered. This was ground in saline-citrate (.1 M each) with Pyrex powder in a conical glass mill (thanks to Burris). After sedimenting the debris, the extract was precipitated with 2 vols. alcohol. The sediment was taken up again in saline, (sol A) and then an aliquot shaken with $CHCl_3$ and AmOH to deproteinize. This was repeated twice. The now clarified supernatant was then treated with 2 vols. alcohol which gave a rapidly ppting. fibrous material which was readily wrapped around a glass rod, and a granular material, which was rejected. The fibrous material was resuspended in NaCl, sedimented with alcohol in a sterile tube, and after standing several hours, taken up again in sterile saline. This is essentially McCarty's procedure, and the fibrous material must have been largely DNA. Both the initial crude extract A and the purified material B were tested for activity on Y-138 (arginine-leucine). The leucine requirement particularly is relatively stable spontaneously. Amounts of extract equiv. about ½g. original paste were added to 10 ml. YB broth, with controls, etc. Some of the controls were contaminated with something that was not coli, but this did not occur in any of the experimental runs, and I think can be ignored. Y138 was grown for 18 h. 37° in the broths, then washed and plated into minimal, arginine, and leucine. Here are some of the results. (dilutions 1,2,3 are 10^{-1}, 10^{-2}, etc. cc of the aqueous suspension ca. 10^9/ml.):

Treatment	Dilution	Minimal	Arginine	Leucine
None	1	0	0	16
	2	0	0	1
	3	0	0	0
None	1	0	ø 1	32
None	1	0	0	46
A (erude extr)	1	0		
"	1	0		
"	1	0		
B	1	0	0	34
	2	0	0	3
	3	0	0	0
B	1	0	3	30
B	1	0	0	45
controls (the goods ones, and as far as coli goes.....		0		

I obviously have to do some more tests on the A-treatments re single-induced reversions. However, this was a lot of plates and more or less an orientation expt. The dilution platings seem to be superfluous in view of the yields in the controls.

More important, we shall need to try to repeat Boivin's own expts. I wrote him rather directly to ask him to send a susceptible C2-R. Without that as a standard we shall be working in the dark.

I still can't see any clear way out of the 1:1 theory contradiction. Now have essentially the same story with maltose. M-108 which I thought first to be Lac$_4$- turns out to be negative on glucose, galactose and fructose. I don't know whether it is a "non-glycolytic" mutant due to a block in the triose---- scheme, or whether the gene may be the one Monod and I have been looking for which controls the hypothetical carbohydrase precursor, It's going to be another tough nut.

How much time are you going to have before starting to close up to leave? Have you had a chance to do any trans, expts., and how did they go??

Sincerely,

Jack